

SUPPLEMENTARY DATA

Immunoelectron Microscopy and Semi-Quantitative Data Analysis.

LNCaP cells were fixed for 1h on ice in PBS buffer (0.1M Na phosphate/chloride, pH=6.8) containing 2% paraformaldehyde. After repeated washes in PBS, cells were centrifuged into 10% gelatin at 37°C for 5min and then transferred onto a slide, left on ice until solidified and then cut into appropriate blocks (<1mm³). The cubes were immersed in sucrose 2.3M overnight at 4°C on a rotating wheel for cryoprotection. Ultrathin cryosections sections were cut (60-90nm thick) and collected onto 200-mesh hexagonal nickel grids. Ammonium chloride was used to quench free aldehyde groups. After blocking non-specific binding with natural goat serum 10% (RT, 30min) and applying the anti-Tip60 primary antibody, immuno-labelling with 10nm gold-conjugated goat anti-rabbit IgG antibodies (1:50, British Biocell International) was performed. Sections were contrasted with uranyl acetate and viewed with a Philips CM100 microscope. Negative control samples were incubated as above except that the primary antibody was omitted. No signal was detected in the negative controls. Fibrillarin was used as a marker of the dense fibrillar component of the nucleolus and exhibited specific staining (data not shown).

The GC of the nucleolus remained generally unlabelled, whereas there was a degree of staining detected in the nucleoplasmic area near decondensed chromatin formations (data not shown). We were not able to observe a difference in Tip60's intra-nucleolar distribution pattern between the cells grown in FM and SDM. Since we were interested in the transitional zone between DFC and FC (see Fig. 4), rather than the well defined individual sub-nucleolar compartments, no further statistical analysis of the data was undertaken to calculate the particle density per compartment. Instead, the number of sections, GC/DFC/FC units (*i.e.* individual FCs with the

surrounding DFC and GC in the form of concentric layers) and particles counted is given in Table S1 and a qualitative rather than a quantitative conclusion is presented.

FIGURE & TABLE LEGENDS

Figure S1

Tip60 localises in the border area between the FC and the DFC (white arrows). Different size nucleoli are shown. Scale=200nm.

Table S1

Each one of the FCs studied along with the surrounding DFC and GC in the form of concentric layers (see Figure 4), was defined as one unit. Overall 30 units in 8 sections were examined in detail and a total of 97 particles were counted. As illustrated, the vast majority of the labelling particles were found to be in the cortical area of the FCs, at the border with the DFC.